Seed germination in *Citrullus lanatus*. Part 3. The possibility of light as an inhibitory factor for germination of seeds within the fruits based on light measurement studies

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The germination of *Citrullus lanatus* seed is under phytochrome control. The optical properties of the fruit of this species indicate that the quality and intensity of light which penetrates the fruit could be an important factor in the inhibition of seed germination within the fruit.

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Die kieming van *Citrullus lanatus*-sade word deur fitochroom beheer. Die optiese eienskappe van die vrugte van hierdie spesie dui daarop dat die kwaliteit en intensiteit van die lig wat die vrug penetreer 'n belangrike faktor in die voorkoming van saadkieming in die vrug kan wees.

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Introduction

Citrullus lanatus is a summer-growing annual, indigenous to Africa. Towards the end of the growing season many fleshy fruits are formed which remain intact for months after abscission. Although these fruits contain more than 95% moisture, by mass, six months after abscission (Botha & Badenhorst 1982), the seeds fail to germinate inside the fruit. Botha & Grobbelaar (1981) have demonstrated the presence of germination inhibitors in *C. lanatus* fruits but whether these actually prevent the germination of seeds within the fruits has not been shown conclusively. On dispersal from the fruit, the seeds of *C. lanatus* germinate only in the dark (Botha *et al.* 1982a). The negative photoblastic nature of the seeds appears to be common among the cucurbits (Koller *et al.* 1963; Noronha *et al.* 1978; Loy & Evensen 1979). The effect of light on the germination of *C. lanatus* seeds used in this study (Botha *et al.* 1982(b)) as well as some other cucurbits is mediated by phytochrome (Yaniv *et al.* 1967; Spruit & Mancinelli 1969; Loy & Evensen 1979). According to Gutterman & Porath (1975) the germination behaviour of the seeds of two *Cucumis* species is related to the light regime under which the fruits are stored. This suggests that light penetrates these fruits.

Because the seeds of *C. lanatus* are negatively photoblastic the possibility exists that light which penetrates the fruit under natural conditions, could play a part in inhibiting germination of seeds within the fruit.

In this study experiments were performed to test whether phytochrome shifts, predicted from theoretical considerations and light measurements could be implicated as a controlling factor of seed germination within fruits.

Material and Methods

Seeds of *Citrullus lanatus* (Thunb.) Matsumura & Nakai were obtained as described by Botha *et al.* (1982a). Only freshly isolated seeds were used throughout. Seeds of *Lactuca sativa* L. cv. Grand Rapids were obtained from Petoseed in Saticoy California, USA, and stored in closed containers at 0 °C.

Seeds were germinated in 9,0-cm Petri dishes, each containing a single sheet of 7,0 cm Whatman No. 1 filter paper, moistened with 5 cm^3 of distilled water. All germination tests were conducted at 27 °C. In experiments with *C. lanatus* 30 seeds and with *L. sativa* 50 seeds were

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used per replicate. At least nine replicates per treatment were used throughout.

In all germination tests with artificial light the primary light source consisted of a combination of 'cool white' fluorescent tubes and tungsten bulbs. Light emitted by this source is designated white light, the spectral composition of which is shown in Figure 1a. In order to obtain light with different spectral compositions Cinemoid filters were used in combination with the white light source. For red rich light one layer each of No. 1 and 14 and for far-red rich light, one layer each of No. 5a and 20 Cinemoid filters were used. Green rich light was obtained by filtering white light through one layer of No. 39 and blue rich light by using one layer of No. 20 Cinemoid.

The spectral optical properties {absorptivity $a(\lambda)$, transmissivity $t(\lambda)$ and reflectivity $r(\lambda)$} of the whole *Citrullus* fruit six months after abscission were measured for the wavelength (λ) range from 400 to 1 350 nm by means of an Isco-SR spectroradiometer (Instrumentation Specialties, Lincoln) and the integrating sphere equipment described by Eller (1972). The spectroradiometer was calibrated with an Isco-SR calibrator (Instrumentation Specialties, Lincoln). The mean optical property $\overline{sp} = \overline{a}$ or $\overline{t}$ or $\overline{r}$ for the whole fruit was calculated by means of the formula

$$\overline{sp} = \{ \int_{\lambda_1}^{\lambda_2} sp(\lambda)\, d\lambda \} / (\lambda_2 - \lambda_1). \quad (1)$$

The transmissivity of the 9-mm layer of fruit tissue (exocarp and mesocarp) surrounding the seedbearing tissue of the fruit was also determined. From this the transmitted radiation inside the *Citrullus* fruit was derived from the equation

$$E_t(\lambda) = E(\lambda) \times t(\lambda). \quad (2)$$

The spectral irradiance $E(\lambda)$ of the light incident on the seeds during the germination experiments in Petri dishes was also determined by means of the spectroradiometer for the wavelength range 400 to 800 nm. The spectral composition of the red, far-red, blue and green light obtained from the fluorescent-tungsten primary light source is shown in Figure 1b. Photon irradiance $E_p(\lambda)$ for the wave range λ_1 to λ_2 was computed from the spectral irradiance values by means of the equation

$$E_p(\lambda) = \{k \times \int_{\lambda_1}^{\lambda_2} E(\lambda) \times \lambda\, d\lambda\} / h \times c_0, \quad (3)$$

where c_0 is the speed of light, h is Planck's constant and k is the unit conversion factor.

The data processing of the measured values of the spectral properties and the irradiance was carried out by the Computation Centre of the University of Zürich where the calculations in connection with the involvement of the phytochrome system were also performed.

Phytochrome system considerations

The phytochrome system consists of two interconvertible pigment forms, one (P_r) absorbing maximally near 600 nm and the other (P_{fr}) near 730 nm. If we assume that only the photochemical transitions $P_r \rightarrow P_{fr}$ and $P_{fr} \rightarrow P_r$ exist and that they obey first-order kinetics, then the equations

$$\frac{dP_r}{dt} = E \times (\alpha_{fr} \times P'_{fr} - \alpha_r \times P'_r) \quad (4)$$

for the transitions $P_{fr} \rightarrow P_r$ and

$$\frac{dP_{fr}}{dt} = E \times (\alpha_r \times P'_r - \alpha_{fr} \times P'_{fr}) \quad (5)$$

for the transition $P_r \rightarrow P_{fr}$, and

$$P'_r + P'_{fr} = P_{tot} \quad (6)$$

are valid (Hendricks, Butler & Siegelman 1962; Holmes & Fukshansky 1979), where P'_r and P'_{fr} are the concentrations of the two forms of phytochrome; E the photon irradiance; α_r and α_{fr} are the action coefficients for the phototransition of P_r and P_{fr} in solution.

$$\alpha_r = \epsilon_r \times \phi_r \quad (7)$$

and

$$\alpha_{fr} = \epsilon_{fr} \times \phi_{fr}, \quad (8)$$

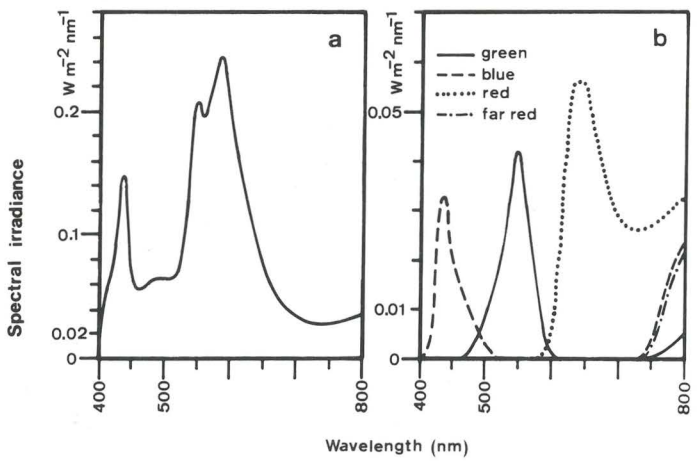

Figure 1 Spectral irradiance of the artificial illumination used for the germination experiments. a: Fluorescent-incandescent primary light source; b: Radiation transmitted through the filter combinations.

where ϵ_r, ϵ_{fr} and ϕ_r, ϕ_{fr} are the extinction coefficients and the quantum yields, respectively.

If we change to molar fractions,

$$P_r = 1 - P_{fr}, \tag{9}$$

and rearrange equations (4) and (5), we obtain

$$\frac{dP_r}{dt} = E \times \{(\alpha_{fr} + \alpha_r) \times P_{fr} - \alpha_r\} \tag{10}$$

and

$$\frac{dP_{fr}}{dt} = E \times \{(\alpha_{fr} + \alpha_r) \times P_r - \alpha_{fr}\}. \tag{11}$$

At the photostationary state (E = constant)

$$P_r \neq P_{fr}. \tag{12}$$

For the purpose of an experimental model, we can assume that at a particular non-photostationary state,

$$P_r = P_{fr}. \tag{13}$$

According to equations (4) and (5), either transformation $P_r \rightarrow P_{fr}$ or $P_{fr} \rightarrow P_r$ will then prevail for a short time until the photostationary state with

$$\frac{dP_r}{dt} = \frac{dP_{fr}}{dt}$$

is re-established.

If we can estimate which transition will prevail at time $+0$ which is the time immediately after we have set $P_r(+0) = P_{fr}(+0)$, then we can deduce which one of the two forms of phytochrome is to be at a higher concentration at the photostationary state, but we cannot state the exact value of their molar fractions. Since we assume

$$P_r(+0) = P_{fr}(+0),$$

we obtain

$$E \times (\alpha_{fr} + \alpha_r) \times P_{fr}(+0) = E \times (\alpha_{fr} + \alpha_r) \times P_r(+0) = \alpha \tag{14}$$

Introducing equation (14) into formulas (10) and (11), we obtain

$$\frac{dP_r(+0)}{dt} = \alpha - (E \times \alpha_r) = f_r(+0), \tag{15}$$

and

$$\frac{dP_{fr}(+0)}{dt} = \alpha - (E \times \alpha_{fr}) = f_{fr}(+0). \tag{16}$$

If

$$E \times \alpha_r < E \times \alpha_{fr}, \tag{17}$$

then

$$f_r(+0) > f_{fr}(+0), \tag{18}$$

and therefore

$$\frac{f_r(+0)}{f_{fr}(+0)} = f\left(\frac{E \times \alpha_{fr}}{E \times \alpha_r}\right) = f\left(\frac{a_{fr}}{a_r}\right), \tag{19}$$

where f denotes molar concentration. For a given spectral distribution of E within the wavelength range from λ_1 to λ_2, we therefore have

$$f\left(\frac{a_{fr}}{a_r}\right) = f\left(\frac{\int_{\lambda_1}^{\lambda_2} E(\lambda) \times \alpha_{fr}(\lambda)\, d\lambda}{\int_{\lambda_1}^{\lambda_2} E(\lambda) \times \alpha_r(\lambda)\, d\lambda}\right), \tag{20}$$

and by calculalting a_{fr}/a_r we can deduce which form of phytochrome shall prevail at a given irradiance $E(\lambda)$. Since we assumed $P_r = P_{fr}$ at time $+0$, we can, in equations (7) and (8), use the molar extinctions of P_r and P_{fr} for ϵ_r and ϵ_{fr} respectively and $\alpha_r(\lambda)$ and $\alpha_{fr}(\lambda)$ would then represent the action spectra given by Butler, Hendricks & Siegelman (1965). If $a_{fr}/a_r > 1$, then P_r will be present in higher concentrations than P_{fr} at the photostationary state at the irradiation $E(\lambda)$. However, should $a_{fr}/a_r < 1$, then the concentration of P_{fr} will be higher than that of P_r.

Results and Discussion

The spectral optical properties of the whole ripe fruit of *C. lanatus* were measured and the results are shown in Figure 2. Also included in Figure 2 is the transmissivity of the outer 9 mm-layer of fruit tissue (exocarp and mesocarp). At this position the first layer of seeds occurs.

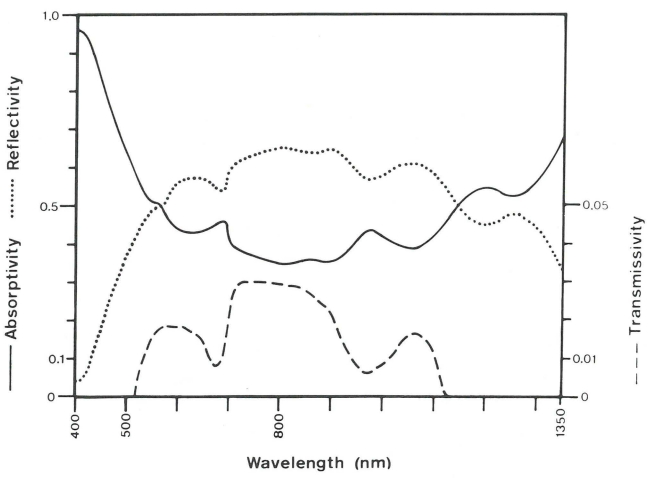

Figure 2 Spectral optical properties of a whole fruit of *Citrullus lanatus* six months after abscission from parent plant. ——— Absorptivity of the whole fruit, ... Reflectivity of the whole fruit, ------ Transmissivity to a position 9 mm inside the fruit.

The optical properties of the fruit are as expected with a high reflectivity of 43,3% in the visible wavelength range. The mean optical properties for different wavelength

Table 1 Mean optical properties of a whole *Citrullus lanatus* fruit and of a piece of the outer 9 mm of the fruit wall six months after the fruit abscissed from the parent plant

	Mean optical property %
Absorptivity whole fruit	
400 . . . 750 nm	56,7
400 . . . 1 350 nm	49.1
Reflectivity whole fruit	
400 . . . 750 nm	43,3
400 . . . 1 350 nm	50,9
Transmissivity to a position 9 mm inside the fruit	
400 . . . 1 350 nm	1,15

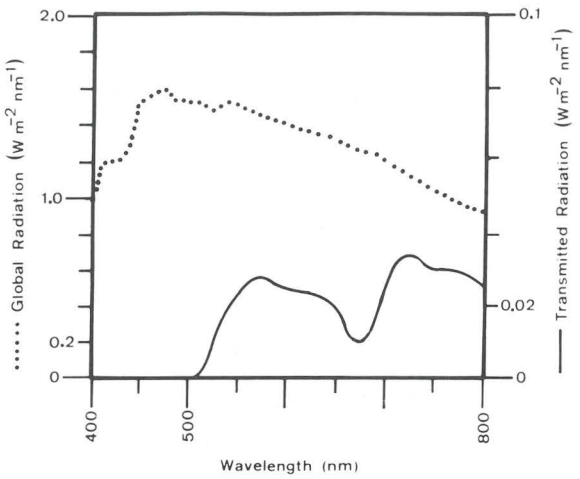

Figure 3 Irradiance inside and outside a fruit of *Citrullus lanatus*. ..... Inciding global radiation in Pretoria (Tvl), 1979.12.08, 11h00, clear sky; ——— Fraction of that radiation transmitted to a position 9 mm inside the fruit.

ranges are given in Table 1.

The spectral global irradiance measured about noon in November in Pretoria (Figure 3) was used to estimate the irradiation of the *Citrullus* seeds inside the fruit at about 9 mm from the fruit surface. The spectral irradiance values in Figure 3 were used, together with the ones presented in Figure 1, to calculate the phytochrome ratios in the seed during the germination experiments and 9 mm inside the fruit during solar radiation.

The results of the germination experiments together with the phytochrome estimations according to equation (20) are listed in Table 2. The predicted domination of either the P_r or the P_{fr} form of phytochrome corresponds very well with the observed germination results of *C. lanatus* and *L. sativa* seeds. The exceptions are in artificial white light and solar radiation. According to the predicted phytochrome shift P_{fr} should prevail in the seeds under both treatments. Lettuce seed germination is high under both treatments as expected, but the germination of *C. lanatus* is strongly inhibited (Table 2). Although the predicted phytochrome shift is towards P_{fr}, white light will result in the formation of a substantial amount of phytochrome intermediates (Kendrick & Spruit 1972). According to these authors more than 30% of the total phytochrome can be maintained as intermediates under incandescent white light. The fraction of phytochrome in the P_{fr} form will thus be considerably smaller under high intensity irradiation than would be expected from calculations of the photostationary state. The obtained P_{fr}/P_{tot} ratio after artificial white light or solar radiation probably results in a P_{fr} concentration which is above the threshold value required for the germination of the positively photoblastic lettuce seeds but which is below the threshold value required by the negatively photoblastic *Citrullus* seeds.

It has been shown that intermittent irradiation is as effective as continuous irradiation in inhibiting *C. lanatus* germination, thereby implying control by a low-energy reaction (Botha *et al.* 1982 a&b). The possibility, that some of the results observed with *C. lanatus* (Table 2) could be due to a high irradiance reaction (Kendrick 1976) cannot, however, be ruled out.

Table 2 Germination of *Citrullus lanatus* and *Lactuca sativa* cv. Grand Rapids seeds under various conditions and the calculated phytochrome form present in highest concentration in the seed in each case

Treatment	a_{fr}/a_r	Form of phytochrome predicted to be in highest concentration	% Germination after 72 h	
			Citrullus	*Lactuca*
In dark	–	P_r or P_{fr}*	94,0± 5,5	4,0±0,5
Artificial illumination (white light in plant growth chamber)	0,53	P_{fr}	17,0± 6,0	92,0±2,5
Green light	0,53	P_{fr}	56,0±15,0	89,0±6,8
Blue light	2,39	P_r	4,0± 4,1	0,5±1,2
Red light	0,62	P_{fr}	48,0±15,0	95,0±4,0
Far red light	>>1	P_r	0,8± 2,4	1,5±1,2
Solar radiation (Fig. 3)	0,71	P_{fr}	8,0± 5,0	64,0±8,0
Transmitted solar radiation inside *Citrullus* fruit	1,1	P_r	none	–

± = standard deviation

* P_r for positive photoblastic seeds and P_{fr} for negative photoblastic seeds (Kendrick 1976)

The calculations for the light transmitted to the seeds inside the fruit indicate that P_r must prevail if the fruits are exposed to sunlight (Table 2 and Figure 3). Although the phytochrome shift in the seeds, while still inside the fruits will be towards P_r formation during solar irradiation, phytochrome intermediates will form. According to Kendrick & Spruit (1972), conditions of pigment cycling as in mixed red/far-red light will result in the accumulation of meta-Rb. This could explain why isolated *C. lanatus* seeds germinate when kept in darkness as meta-Rb is converted to P_{fr} in the dark (Kendrick 1976). The form in which phytochrome is present in highest concentration in the seeds under natural conditions is thus controlled by the light filtering capacity of the fruit. Consequently we deduce that light penetrating the fruit of *C. lanatus* could be an important factor in inhibiting the germination of the ripe seeds inside the fruit.

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